



Dynamics of soil dissolved organic carbon pools reveal both hydrophobic and hydrophilic compounds sustain microbial respiration



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ARTICLE INFO

Article history:

Received 19 June 2014

Received in revised form

26 August 2014

Accepted 2 September 2014

Available online 19 September 2014

Keywords:

Dissolved organic carbon

Compost

Soil respiration

Humic acids

Fractionation

ABSTRACT

The quality of dissolved organic carbon (DOC) released from soil organic amendments may influence soil microbial activity and the quality of the soil's DOC pools. Measurements of total DOC are often considered in relation to microbial activity levels but here we propose that quantification of DOC fractions is a more informative alternative. In a laboratory incubation, soil received DOC that was extracted from three organic matter sources: fresh compost, mature compost, and a mixture of the two. Soil microbial respiration (CO₂ emission), and concentrations of hydrophobic (humic acids (HA), fulvic acids (FA) and neutrals (HoN)) and hydrophilic (Hi) DOC fractions were measured throughout the 35 d incubation. The A₂₅₄ specific UV absorption of total and HA DOC were measured at the start and end of the incubation as an indicator of aromaticity. Microbial respiration rates were highest in soils amended with fresh compost DOC, which had a higher proportion of Hi compounds. Concentration of Hi was significantly and positively correlated with soil respiration, explaining 24% more variation than total DOC. Humic acid concentrations significantly decreased over 35 d, including a 33% reduction in HA from an unamended control soil. Compost treated soils' HA pools increased in aromaticity, suggesting preferential mineralization of the least aromatic HA molecules. A decrease in SUVA₂₅₄ values in other HA pools may be the result of HA degradation in the absence of low-aromatic HA. Our observation of depletion of hydrophobic compounds from the HA fraction provides evidence that humic substances can be a relatively reactive pool, which can provide, together with hydrophilic compounds, a readily available C source to the microbial community.

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1. Introduction

Dissolved organic carbon (DOC) plays a key role in sustaining soil microbial activity due to its solubility and lability (Kalbitz et al., 2000; Chantigny, 2003) and thus may be a pertinent indicator of soil quality. Soil DOC is both a substrate for microbial activity and a byproduct of the subsequent microbial metabolic processes (Marschner and Kalbitz, 2003; Van Hees et al., 2005; Bolan et al., 2011). The quality of DOC influences the variability of several soil factors, such as microbial community composition (De Graaff et al., 2010), nutrient availability and leaching (Ros et al., 2010), and the

rate of soil C turnover (Jandl and Sollins, 1997; Van Hees et al., 2005; Boddy et al., 2007). As a substrate, DOC may originate from plant residues, root exudates, decomposing litter, and, in agricultural soils, from applied organic amendments (Chantigny, 2003). The range in potential sources results in a biochemically heterogeneous DOC solution, which exacerbates uncertainty in predicting how a particular amendment impacts soil microbial activity and C turnover rates.

Several studies have measured the influence of various qualities of C additions on soil C turnover (Guggenberger et al., 1994; De Nobili et al., 2001; Kalbitz et al., 2003a; Boddy et al., 2007). Those describing rapid evolution of CO₂ from soil (<1 h after C addition) attributed this to the turnover of low molecular weight (LMW) C compounds (De Nobili et al., 2001; Boddy et al., 2007), such as short-chain polysaccharides and amino acids. Van Hees et al. (2005) compared forest soil respiration rates between LMW and high molecular weight DOC compounds and concluded that the

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latter drives only 14% of CO₂ emissions. This suggests that DOC containing high proportions of LMW compounds would stimulate microbial activity relatively more than DOC with a smaller proportion of LMW C. Qualifying DOC into proportions of labile, LMW C vs more recalcitrant, aromatic C compounds may therefore provide a more powerful indication of C turnover rates and microbial activity potential than measurements of DOC concentration alone.

One method of qualifying DOC is to partition the total pool into operationally defined fractions based on their solubility and relative hydrophobicity (Van Zomerem and Comans, 2007). This is a rapid-batch procedure based on the International Humic Substances Society's standard method for isolation of humic substances from organic matter in soils and natural waters (Swift, 1996; Thurman and Malcolm, 1981). By equilibrating a DOC solution with an absorbent hydrophobic resin (DAX-8), hydrophobic compounds are pulled out of solution and physically separated from hydrophilic compounds. The isolated pools resulting from this fractionation are distinguishable as aromatic, hydrophobic fraction (humic (HA) and fulvic acids (FA), hydrophobic neutrals (HoN)) and a hydrophilic fraction (Hi). Despite the relative ease of the method and its pervasiveness in environmental chemistry applications, only a few studies have looked at the dynamics of DOC quality over time using these fractions as a proxy. Ros et al. (2010) quantified fractions in three different soils after 35 d of incubation at different temperatures. They observed a decrease in the absolute concentration of a sandy soil's HA fraction at 10 and 20 °C, which resulted in this pool contributing a smaller proportion of the total DOC. At the same time, this study observed a stable Hi concentration at the end of incubation, which seems counterintuitive in contrast with other studies describing a high degradability of LMW compounds (De Nobili et al., 2001; Boddy et al., 2007; Bolan et al., 2011; Lannan et al., 2013) which would be present in the Hi pool. In a study of how known compounds in homogenous, prepared solutions are partitioned between hydrophobic and hydrophilic pools, Amery et al. (2009) observed the majority of aliphatic C and 97% of a fractionated glucose solution was measured in the hydrophilic pool. This pool contains the largest proportion of LMW compounds (<500 Da) (Thurman et al., 1982). Conversely, hydrophobic HA molecules are considered a source of C quite recalcitrant to microbial degradation and thus one of the most stable soil organic compounds (Kalbitz et al., 2003b). This HA pool also contains larger, more aromatic molecules, in the range of 500–10,000 Da (Thurman et al., 1982). Fulvic acids tend to be less aromatic and lower in molecular weight (500–2000 Da (Thurman et al., 1982)) than humic substances. Guggenberger et al. (1994) have proposed that hydrophobic compounds may be in an intermediary state of decomposition between DOC source material and Hi fractions, but the authors do not identify a mechanism behind this flux or explore the implications for soil quality.

The biological relevance of each of these DOC fractions in soil remains unclear, especially for agricultural soils (Haynes, 2005), as research has yet to link fractionation in real-time with measurements of microbial activity. Although Chantigny (2003) describes the effect of organic amendments on soil DOC concentration, their impact on soil DOC quality over time may be highly variable depending on the amendment's composition and their effect on C turnover rates. The aim of this study was to investigate the dynamics of soil DOC quality using fractionation and specific UV absorbance, in parallel with measurements of soil microbial respiration, in a soil amended with compost-derived DOC. Due to increased bioavailability, we hypothesized that soils receiving DOC with a higher proportion of hydrophilic compounds would have respiration rates stimulated more so than those receiving more aromatic hydrophobic compounds. We expected DOC extracted from soil to reflect the characteristics of the added DOC with respect to prevalence of

certain fractions and aromaticity measurements. These quality measurements and soil microbial respiration rates were carried out simultaneously, in order to elucidate the influence of hydrophobic vs hydrophilic DOC fractions on soil C turnover rates and better understand the contribution of DOC to soil activity levels.

2. Materials and methods

2.1. Amendment characterization

Two different composts were used as a source of DOC to add to soil. To obtain DOC varying in quality, the composts were selected based on their different source materials and maturation treatments. They were collected in October 2012 from two different commercial compost facilities in the Netherlands. The first compost, hereafter referred to as *fresh compost*, was collected from the Orgaworld Biocel fermentation facility in Lelystad, The Netherlands. The input material of the fresh compost consisted of municipally collected household fruit, vegetable and garden residues. These materials were composted for 1 d above 65 °C (internal temperature of the compost heap) and 6 d above 45 °C, with two instances of the material being turned. The second compost, hereafter referred to as *mature compost*, was collected from the Van Iersel compost facility in Biezenmortel, The Netherlands. It consisted, by mass, of 75% soil sieved from woody compost inputs and 25% municipal grass cuttings. The composting period for the mature compost lasted for 2 weeks at an internal temperature of 60–70 °C, and the compost heap was turned twice a week. After this period, the mature compost was cured at ambient outdoor temperatures for 6–8 weeks. Both composts were collected at the stage of being a final, commercial product and their chemical characteristics are described in Table 1.

The compost DOC was obtained via a 1:2 compost to ultra-pure water (UPW) extraction: after 1 h equilibration by horizontal shaking, 20 min centrifuging at 3000 g and 10 min ultra-speed centrifuging at 11,700 g, the supernatant was vacuum-filtered through a 0.2 µm cellulose nitrate membrane, which had been pre-rinsed with 100 ml UPW to avoid C release from the membrane itself (Khan and Subramania-Pillai, 2006). The filter size was selected to exclude any microbes from the solution (Norris and Ribbons, 1969). This extraction was performed on both fresh and mature compost, as well as a 50/50 mass mixture of the two (hereafter referred to as *mix compost*) so that three DOC amendment solutions ranging in quality were prepared. The solutions were then freeze-dried for stable storage and to allow for redissolution at equal DOC concentrations.

2.2. Soil collection and characterization

The soil was collected in October 2012 from the Wageningen UR Applied Plant Research site in Vredepeel, Netherlands, from the 0–20 cm layer of an agricultural field. It is classified as non-calcareous loamy sand soil. It was suitable to assess DOC dynamics, since a high level in carbonates and clay minerals strongly affects DOC concentration and adsorption (Kalbitz et al., 2000; Chantigny, 2003), and contribute to abiotic soil CO₂ release; this soil was less than 0.01% CaCO₃ (Table 1) and only 1% clay. Roots and crop residues were manually removed and the soil was air dried at 22 ± 1 °C for 8 h until 30% water holding capacity was reached.

2.3. Soil incubation and respiration measurements

An incubation experiment was established in November 2012 to measure DOC turnover in soil to which the extracted compost DOC had been added. The incubation was conducted in 575 ml glass bottles with 150 g dry-weight-equivalent soil at 20 °C in the dark

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